Chlorine Dioxide

Toxicity in Animal Experiments and Industrial Risks

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Introduction

Chlorine dioxide gas has excited increasing attention since the discovery of its industrial usefulness for bleaching *inter alia* paper pulp. This has entailed interest in the toxicology of the gas. The available industrotoxicologic literature, however, contains only a few reports on chlorine dioxide. Elkins [1] described two cases of chlorine dioxide poisoning, one of them fatal, after exposure to about 19 ppm for a not precisely defined period. According to this writer, the gas is a respiratory irritant and must be handled with extreme caution.

The maximum allowable concentration of chlorine dioxide has been estimated at 1 ppm. A search of the literature, however, has yielded only one mention of tests on animals. Taylor and associates [2] reported figures for the duration of exposure to, and concentration of, chlorine dioxide in experiments on guinea pigs but presented no detailed data.

The chemical and technical literature on the subject is more abundant, e. g., Holst.[3] For present purposes, however, the only relevant aspects are laboratory production and determination of chlorine dioxide gas.

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Production of Chlorine Dioxide

In gaseous form chlorine dioxide (ClO_2) is yellow and has an unpleasant, irritating, and characteristic odor. Its melting point is −59 C, and its boiling point is +11 C. Liquid ClO_2 is red and explosive. The gas is also explosive, although this property seems to have been exaggerated by earlier writers. According to Angel,[4] the explosion level is 10% of ClO_2 in a gaseous mixture.

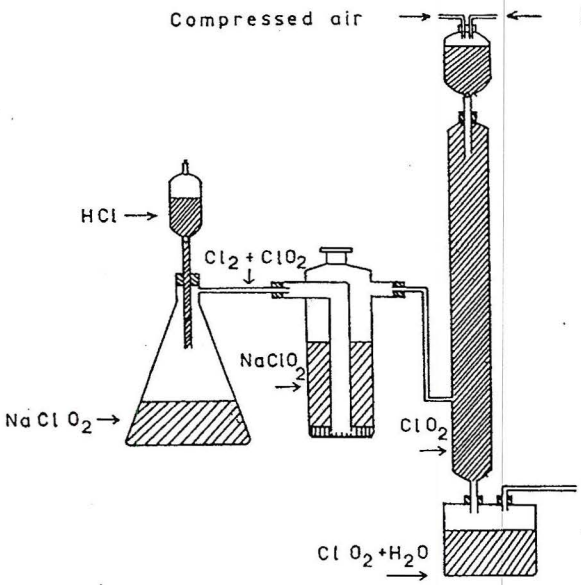

Figure 1

When chlorine dioxide comes into contact with organic matter, however, even very low concentrations carry a risk of explosion. This fact should be kept in mind when the gas is used.

For the animal experiments in this study chlorine dioxide was produced with the apparatus shown in Figure 1 and according to the following mode of reaction:

$$8NaClO_2+8HCl\rightarrow 8NaCl+Cl_2+4H_2O+6ClO_2 \quad (1)$$

$$2NaClO_2+Cl_2\rightarrow 2NaCl+2ClO_2 \quad (2)$$

Hydrochloric acid was run into a flask containing solid sodium chlorite (Equation 1). The chlorine gas formed and the chlorine dioxide gas were led into a wash bottle containing sodium chlorite solution (Equation 2). The chlorine gas reacted with this solution to form chlorine dioxide. In the dissolving column the chlorine dioxide was then dissolved in distilled water according to the countercurrent principle. This method thus avoided the presence of chlorine gas, which is potentially important for measurements of chlorine dioxide concentration.

Such determinations were made with the titrimetric procedure described by Giertz.[5] The iodometric principle may briefly be described as follows:

A solution of potassium iodide is buffered with phosphate to pH 7.0, checked with litmus paper. ClO_2 reacts with KI in neutral solution thus:

$$ClO_2+KI\rightarrow KClO_2+\tfrac{1}{2}\ I_2.$$

Any Cl_2 gas present reacts with the dissolved KI in the following manner, whether the solution is neutral or acid:

$$Cl_2+2KI\rightarrow 2KCl+I_2.$$

If the pH is somewhat alkaline, the chlorine may react by forming iodate, which distorts Cl_2 readings.

Acid pH prevents this iodate formation but entails a risk that the chloric acid formed by the ClO_2 will have time to react. For further details of the method the reader is referred to Giertz.[5]

In determining the content of ClO_2, calculation is first made of the content of I_2 at pH 7.0. Titration is carried out with $Na_2S_2O_3$ of suitable normality (0.1 or 0.01 N). The sample is then acidified, and after three minutes the following reaction is considered to be complete:

$$KClO_2+4KI+2H_2SO_4\rightarrow 2I_2+2K_2SO_4+KCl+2H_2O.$$

The quantity of $Na_2S_2O_3$ now required for titration is equivalent to four-fifths of the ClO_2 gas. As the KI solution is somewhat unstable, it is exposed as little as possible to light.

On the basis of the above reactions, Giertz[5] evolved the following formulae for calculating ClO_2:

Tn=amount of $Na_2S_2O_3$ required at neutral pH.

Ts=amount required at acid pH (i. e., including Tn).

N=normality of the $Na_2S_2O_3$ solution.

The amount of ClO_2 is thus $5/4\ (Ts-Tn)$, and the amount of Cl_2 is $Ts\ 5/4(Ts-Tn)$. ClO_2 undergoes a change in valency of five, ($Cl^{4+}+5e\rightarrow Cl-$), whereby its gram equivalent becomes $\frac{67.5}{5}$ and the number of milligrams of ClO_2

$$\frac{5\cdot(Ts-Tn)N\cdot 67.5}{4.5}=\frac{5}{4}\cdot(Ts-Tn)N\cdot 13.5.$$

As 1 liter of ClO_2 weighs $\frac{67.5}{22.4}$ gm. 1 ppm corresponds to $ClO_2=\frac{67.5}{22.4}\cdot 10^6=0.003$ mg/l.

The pure solutions of ClO_2 used for the experiments on animals made measurement of chlorine concentration superfluous. In industrial premises, however, it may be necessary to distinguish between Cl_2 and ClO_2. The above reaction should therefore be kept in mind.

Animal Experiments

The paucity of investigations of ClO_2 toxicity in animals seemed to warrant study of the effects of respiratory exposure to the gas.

The apparatus used is illustrated in Figure 2. Into the mixing vessel were led compressed air alone and ClO_2 gas via air bubbled through a solution of ClO_2. After passing through the exposure chamber, the bottom of which was covered with blue gel, the air-ClO_2 was allowed to escape either through a fume cupboard or, when the mixture was sampled, through a Peligot tube containing KI solution and via a drying tower through a gas meter.

Four series of rats were exposed to ClO_2 gas in various concentrations and for various periods.

The first series comprised six rats. Three of these were thrice exposed at weekly intervals to very high ClO_2 concentrations for three minutes. The other three rats acted as controls. The results are presented in

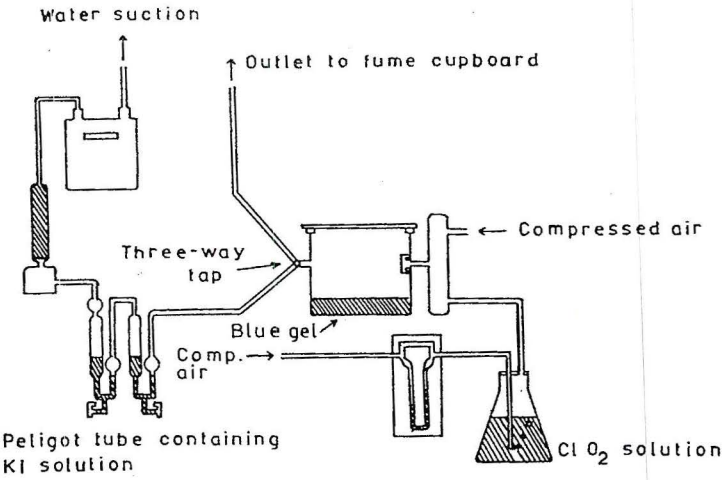

Figure 2

Table 1. The respective approximate concentrations of ClO_2 were 3400, 1100, and 800 ppm. Despite the short period of exposure the rats were clearly affected. Respiratory distress was conspicuous. The weight of the three exposed rats fell, while that of the three controls increased.

TABLE 1.—*Weight of Rats Exposed to ClO_2 for Three Minutes at Weekly Intervals*

Exposed on, Day	ClO_2, Ppm	Mean Weight, Gm.	
		Exposed Rats	Controls
1	3,435	268	247
8	1,118	262	257
15	760	242	269

Histologic study showed small areas of recent bronchopneumonia and hyperemia of the renal corticomedullary junction in two of the exposed rats. The lungs and kidneys of the third exposed rat were normal.

In all three control rats the lungs were healthy, but renal hyperemia was present in two.

The four rats of the second series were exposed to about 260 ppm of ClO_2 for two hours. To maintain a fairly constant concentration, the gas was changed every half hour. The readings were 265, 264, 266, and 245 ppm, respectively.

The rats were severely affected. Ocular discharge and epistaxis were specially prominent. One rat died after about an hour's exposure. The others were killed as soon as exposure had ceased. The kidneys, liver, spleen, and lungs of all four rats were microscopically examined. Pulmonary edema and circulatory engorgement were invariably present.

The third series consisted of 10 rats. Five were exposed daily for a mean period of four hours to about 10 ppm of ClO_2. The other five were used as controls. Table 2 shows the concentration range of ClO_2 for each day. The urine was examined for protein, and the weight of the rats was charted. The exposed rats showed marked distress in the form of rhinorrhea and embarrassed respiration.

The number of positive urinary protein tests was not greater in the exposed rats than in the controls. The weight of the exposed rats, however, showed a mean re-

TABLE 2.—*Weight of Rats Exposed to ClO_2 for About Four Hours Daily*

Day of Exposure	ClO_2, Ppm (Range)	Mean Weight, Gm.	
		Exposed Rats	Controls
1	2.2— 4.7	287	289
3	7.3—14.5	271	290
4	8.6—11.4	273	291
7	7.1—12.4	254	287
8	9.5—13.8	248	287
9	6.0—12.1	229	288
10	9.2—12.3	221	292
11	2.3— 6.6	217*	295§
13	8.9—10.3	213†	298 ‖
14		201‡	301 ¶

*One rat died.
†Two rats died.
‡Two rats died before exposure commenced.
§One rat killed.
‖ Two rats killed.
¶Two rats killed.

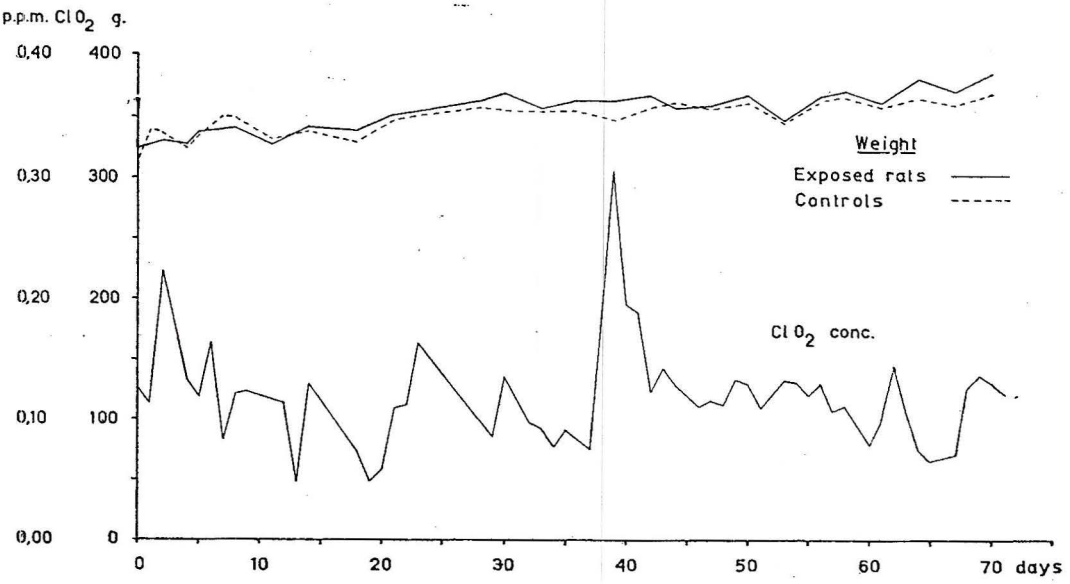

Figure 3

duction of about 80 gm., while that of the controls was largely unaltered.

One rat died after 10 days' exposure, and two died after a further day. The two remaining rats died on the 13th day.

The kidneys, liver, and lungs were examined. In all the exposed rats microscopy showed changes resulting from respiratory infection with acute renal and hepatic congestion. Nothing of note was seen in the control rats.

The fourth series also contained 10 rats. Five were exposed during about 10 weeks to approximately 0.1 ppm of ClO_2 for a mean daily period of 5 hours. The other five rats were controls (Fig. 3).

During the period of exposure the rats were not noticeably affected. Nor did their weight curve differ from that of the controls.

Histologic examination showed normal kidneys, liver, and lungs in the exposed rats. Respiratory infection was present in three of the control rats.

The effect of the exposure procedure as such was investigated by exposing six rats to compressed air without ClO_2 for 5 hours daily during 10 days. The rats' condition and weight remained unaffected (Table 3).

Histologic study of these six rats revealed purulent bronchitis in two. The lungs of the others were normal in appearance, and in no case was there renal hyperemia.

Industrial Investigations

As already mentioned, ClO_2 is increasingly being employed as an industrial bleaching agent. The risk of toxicity has thereby also increased. The exposure to ClO_2 in two large industrial plants was therefore investigated.

In a paper pulp works a total of 10 air samples was taken in the preparation department and in the bleachery. The apparatus used is shown in Figure 4. Air was sucked through a wash bottle containing KI solution and was conducted via a drying tower to a gas meter. In 9 of the 10 samples

TABLE 3.—*Weight of Rats Exposed to Compressed Air*

Rat, No.	Weight, Gm.		
	Before Exposure	After Exposure	Difference
1	194	221	+27
2	214	228	+14
3	202	225	+23
4	205	226	+21
5	220	227	+ 7
6	190	208	+18
Mean	204	222	+18

2% (range ±0-−3.9). The corresponding figure for Cl_2 was also 2% (range +9.9-−2.1). I made no further tests of the method as such, as the above figures show that the differences in the relevant gas concentrations were well demonstrable with Giertz' technique.

Rats were exposed to ClO_2 in various concentrations and for various periods. It was thereby shown that high concentrations produced marked effects even when given for a short time (three minutes or two hours). The rats' respiration was distressed, and there was copious ocular and nasal secretion. Epistaxis was also seen. Necropsy showed recent bronchopneumonia and pulmonary edema in the exposed rats. Circulatory disturbances of congestive type were frequently found. Two of the nineteen control rats, however, also showed circulatory congestion. Five had respiratory infection. Repeated exposure to ClO_2 in high concentration (10 ppm) resulted in death of all the rats within 14 days. Distressed breathing, increased secretion from the nose and eyes, and loss of weight occurred as in the other series of exposed rats. The histopathologic findings were uniform—severe purulent bronchitis, disseminated areas of recent bronchopneumonia, and acute renal and hepatic congestion.

In rats repeatedly exposed to a much lower concentration of ClO_2 (0.1 ppm), no abnormal reaction was detected during life or at necropsy. There was no loss of weight.

In two factories air was analyzed. The concentration of ClO_2 in the great majority of the samples was less than 0.1 ppm. Only in special processes or under "disaster conditions" were higher concentrations registered. The symptoms described by workers who in all probability had been exposed to ClO_2 in toxic concentrations tallied well with the findings in the exposed rats. Thus, there was respiratory distress with "bubbling" breath sounds and increased secretion from the nose and eyes.

These experimental and industrial studies showed, therefore, that the main disorders resulting from exposure to ClO_2 were difficulty in breathing and nasal and ocular discharge. Pulmonary edema and bronchopneumonia were the most important necropsy observations.

In considering a maximum allowable concentration for ClO_2, the following facts are pertinent.

Rats repeatedly exposed to about 10 ppm of the gas showed the above-mentioned reactions and died after less than 14 days' exposure. Repeated exposure to 0.1 ppm for about 10 weeks, on the other hand, produced no injurious effects in rats.

In the industrial premises examined, the atmospheric concentration of ClO_2 seldom reached 0.1 ppm. Men exposed to this concentration appeared to suffer no ill effects. The symptoms reported by men who occasionally had been exposed to higher ClO_2 concentrations, however, were in conformity with the observations in rats.

These results indicate that 0.1 ppm at present is acceptable as a maximum allowable concentration for ClO_2. A study of persons exposed to ClO_2 would nevertheless be of value. It would make for greater clarity in the choice of an M. A. C. based on experimental and clinical considerations.

Summary

Rats were exposed to chlorine dioxide gas in various concentrations and for various periods. Respiratory distress and increased secretion from the eyes and nose resulted. All the rats exposed daily to 10 ppm of ClO_2 died in less than 14 days. Purulent bronchitis and disseminated bronchopneumonia were found at necropsy. No such changes were demonstrable in rats exposed to approximately 0.1 ppm for about 10 weeks.

Air samples from industrial premises very rarely contained more than 0.1 ppm of ClO_2. Men exposed to this concentration were symptom-free. Toxic concentrations, however, in all probability had been reached

on two occasions. The symptoms in the affected men resembled the changes observed in rats exposed to the same gas and included respiratory distress, "bubbling" sounds in the chest, and increased nasal and ocular secretion.

National Institute of Public Health.

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